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SIGNALLING BY RECEPTOR TYROSINE KINASES[1]

Wendy J. Fantl, Daniel E. Johnson, and Lewis T. Williams
Howard Hughes Medical Institute, Program of Excellence in Molecular Biology, and Cardiovascular Research Institute, University of California, San Francisco, California 94143-0724

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CONTENTS

[1]Abbreviations used: RTK, receptor tyrosine kinase; ^{P}Y, phosphorylated tyrosine; PI, phosphatidylinositol; SH2, src homology region; PLC-γ, phospholipase C-γ; GAP, GTPase-activating protein; ERK, extracellular regulated kinase; GNRP, guanine nucleotide releasing protein; PCR, polymerase chain reaction.

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INTRODUCTION

The phenotypic state of a cell is constantly influenced by a variety of extracellular signals. Polypeptide growth factors represent a group of extracellular signals that are critically important for influencing a diverse array of cellular responses including proliferation, differentiation, and cell survival. The effects of many growth factors are known to be mediated by high-affinity receptor tyrosine kinases. Within the past decade, several distinct subfamilies of RTKs have been identified (Figure 1). The members of a given subfamily share common structural features that are distinct from those found in other subfamilies.

Despite the diversity of RTKs, there is a great degree of commonality in the types of intracellular signalling pathways initiated by these proteins. In mammalian systems, biochemical and molecular genetic analyses have shown that for all RTKs, the binding of ligand to the extracellular domain activates the tyrosine kinase in the cytoplasmic domain. This leads to downstream activation of a number of common signalling molecules. Frequently activated proteins include phospholipase C-γ, phosphatidylinositol 3-kinase (PI3-kinase), GTPase-activating protein, pp60c-src, p21ras, Raf-1 kinase, ERK 1 and ERK 2 kinases (also referred to as MAP kinases), and S6 ribosomal kinases. Ultimately, the activation of signalling pathways involving these molecules leads to changes in gene expression and a change in the phenotypic state of the cell. Interestingly, a single type of RTK can elicit very different biological responses in different cell types.

The field of signal transduction by RTKs has expanded dramatically within the past five years. Therefore, it is not possible to cover this area exhaustively. The focus of this review is on receptors. Excellent reviews of the signalling molecules, PI3-kinase (1, 2), PLC-γ (3), Raf (4), and ERK kinases (5) have been recently published. Also, we do not discuss the modification of cytoskeletal proteins in response to growth factors (6). We include information on p21ras because this field has progressed rapidly during the past year. The review is organized into a section on general approaches to studies of receptor-mediated signal transduction, with an emphasis on genetic and biochemical studies, followed by a brief discussion of each subfamily of receptors emphasizing that group's idiosyncrasies.

GENETIC SYSTEMS FOR STUDIES OF SIGNAL TRANSDUCTION

The activation of a common set of signalling molecules has been independently verified through genetic analyses in *Caenorhabditis elegans* and *Drosophila melanogaster*. These studies have shown that RTK molecules are important for the developmental specification of cell types, and that RTKs in these

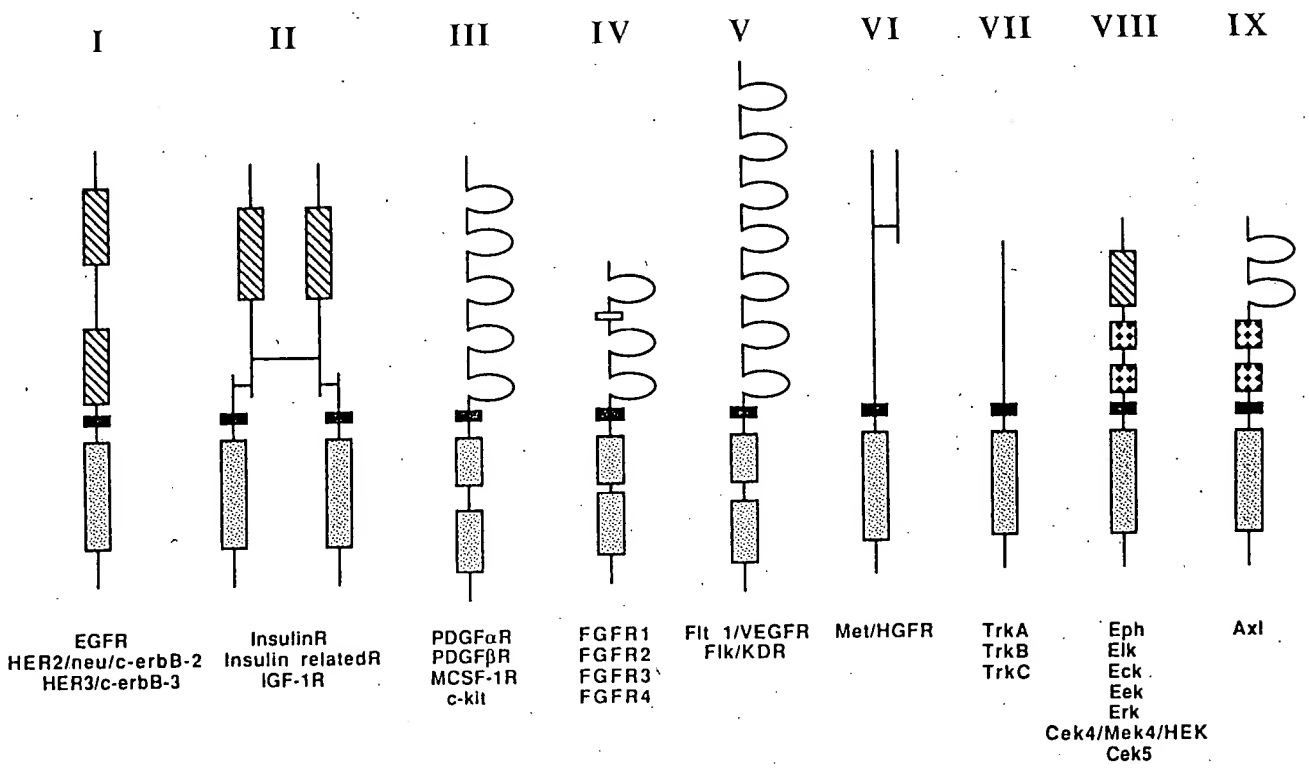

Figure 1 Vertebrate receptor tyrosine kinases. The figure shows distinct subfamilies of RTKs based on the classification proposed by Ullrich & Schlessinger (79). The following structural features are identified: tyrosine kinase domains (stippled boxes), transmembrane domains (solid boxes), cysteine-rich domains (striped boxes), immunoglobulin-like domains (semi-circles), acid box domain (open box), and fibronectin type III domains (checkered boxes). The tyrosine kinase domains of Class III, IV, and V receptors are interrupted by kinase insert sequences.

organisms initiate signalling pathways that are strikingly similar to those found in mammalian systems. In *C. elegans* for instance, the products of the *lin-3*, *let-23*, and *let-60* genes represent structural homologs of epidermal growth factor/transforming growth factor-α, the epidermal growth factor receptor, and ras, respectively, and all are known to be important for proper vulval development (7–9). Genetic studies have shown that *let-60* (ras) is epistatic to *lin-3* (EGF/TGF-alpha) and *let-23* (EGFR), indicating that it is located downstream in the signalling pathway. The product of the *Let-60* gene appears to be downstream from sem-5, an SH2/SH3-containing (src homology region) protein (8, 10).

In *Drosophila*, development of the R7 photoreceptor is dependent on the function of the *sevenless* gene product, a RTK that is structurally related to c-ros and members of the insulin receptor family (11–13). Located downstream from sevenless are several signalling molecules with known vertebrate homologs, including son of sevenless (SOS) protein, Gap1, Rap1, and ras1 (11–13). The SOS protein is homologous to guanine-nucleotide exchange factors and may function to activate ras1. Gap1 and Rap1 probably act to regulate ras1 signalling negatively, consistent with the known biological activities of their vertebrate counterparts in suppressing ras function. Finally, the *Drosophila torso* gene product, a RTK related to the platelet-derived growth factor (PDGF) receptor family, and D-raf, the homolog of mammalian c-Raf1, are important for development of the anterior-posterior axis in the fruit fly embryo (14–16). D-raf is epistatic to torso, indicating that, similar to the location of c-Raf1 in mammalian signalling pathways, D-raf is located downstream of the RTK molecule. Corkscrew, an SH2-containing phosphatase protein, is also epistatic to torso (17).

The combination of genetic and biochemical approaches has presented a clear picture that signalling by different RTK molecules frequently leads to the activation of a common set of signalling molecules. This occurs in a variety of different cell types and organisms. In this review, we focus on paradigms of RTK-mediated signal transduction and the involvement of commonly activated signalling molecules in these pathways.

RECENT BIOCHEMICAL AND CELLULAR STUDIES OF SIGNAL TRANSDUCTION

Interactions Between Receptor Tyrosine Kinases and Signalling Molecules: Binding of Phosphotyrosine to SH2 Domains of PI3-Kinase, PLC-γ, and GAP

Upon binding ligand, RTKs phosphorylate themselves on tyrosines, a process commonly termed autophosphorylation. In the case of some RTKs, ligand binding is known to induce receptor dimerization (18, 19). Following

autophosphorylation, individual phosphotyrosine residues located in the cytoplasmic domains of receptors serve as highly selected binding sites that interact with specific cytoplasmic molecules. These signalling molecules mediate the cellular responses to growth factors. The first clue that signalling molecules associate directly with RTKs and that this association involves a specific domain of the kinases came from the observation that autophosphorylated PDGF β-receptor binds the enzyme phosphatidylinositol 3-kinase (PI3-kinase) (20). A mutant PDGF β-receptor that had a segment of the cytoplasmic region deleted, termed the kinase insert region, did not bind PI3-kinase even though the mutant receptor had tyrosine kinase activity and was capable of activating another signalling molecule, phospholipase C-γ (20). Through a series of mutagenesis studies, it was shown that PI3-kinase can bind to either or both of two distinct sites in the kinase insert region, tyrosines 708 and 719 (mouse β-receptor) (21–24). Another signalling molecule, GTPase activating protein (GAP), binds to tyrosine 739 (Figure 2) (21, 22, 25). Two tyrosines that bind PLC-γ are at positions 977 and 989 (J. A. Escobedo, L. T. Williams, unpublished; 27, 28).

Synthetic peptides representing the sites on the receptor that interact with signalling molecules have been extremely useful in demonstrating the structural basis for the specificity of the interaction between RTKs and signalling molecules. Tyrosine-phosphorylated peptides as short as five amino acids can selectively block the interaction of some signalling molecules with RTKs by occupying the sites on the signalling molecules that recognize receptor sequences. The binding of peptide to the signalling molecules is highly specific

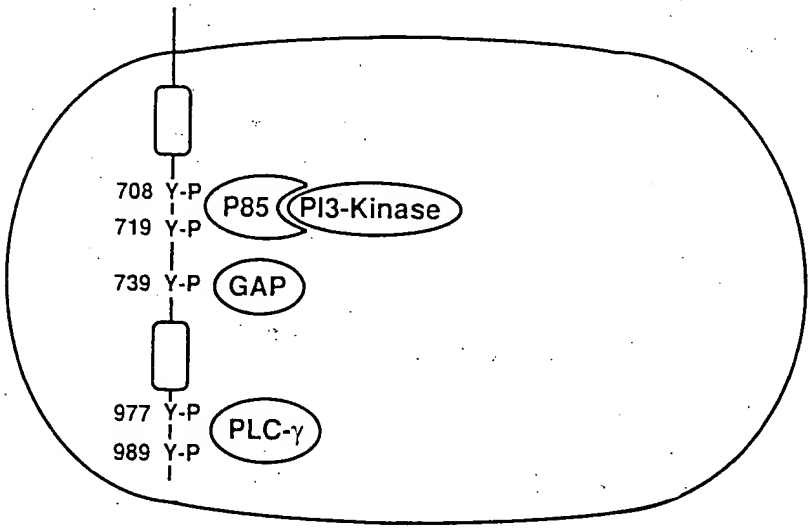

Figure 2 Association of intracellular proteins with the cytoplasmic region of the PDGF receptor. The numbers refer to amino acid positions in the murine PDGF β-receptor.

and occurs at low concentrations of peptide (1–10 μM), but only when the peptides are phosphorylated on key tyrosine residues. For example, the sequences of the PDGF β-receptor at its two binding sites for PI3-kinase are ^{P}YV-P-M-L and ^{P}YM-D-M-S, respectively. Synthetic peptides consisting of either of these sequences block binding of PI3-kinase to the receptor, but do not block binding of either GAP or PLC-γ. Shorter versions of these peptides do not block binding, suggesting that five amino acids is the minimum length of peptide required for a high-affinity interaction with PI3-kinase (22). The common feature of these peptides and the binding site for PI3-kinase on the middle T-antigen protein is the presence of a methionine residue three residues on the carboxyl-terminal side of phosphotyrosine (2, 22, 23). The importance of this motif (YXXMX) as a binding site for PI3-kinase has recently been demonstrated (22), and it was found that only certain amino acids could be substituted at the "unspecified" positions (Xs) of this motif. Phosphotyrosine-containing peptides representing distinct binding sites for GAP or PLC-γ have been used to identify the regions of the PDGF and fibroblast growth factor (FGF) receptors that bind these signalling molecules. From these studies, one can conclude that short sequences flanking receptor phosphotyrosines determine the remarkable specificity of the interaction between signalling molecules and RTKs. The residues on the carboxyl-terminal side of the phosphotyrosine appear to be more important in determining the affinity and specificity of the interaction than residues on the amino-terminal side of the phosphotyrosine.

A common feature of many signalling molecules that are known to bind to RTKs with high affinity is that they contain SH2 domains. SH2 domains are regions of about 100 amino acids that are homologous to the noncatalytic region present in the c-src proto-oncoprotein (29–31). Hanafusa and coworkers (32) first demonstrated that crk SH2 domains can bind directly to tyrosine-phosphorylated proteins in transformed cells. Recently, other investigators have shown that SH2 domains will also bind to tyrosine-phosphorylated receptors. The structures of several SH2 domains have been determined by nuclear magnetic resonance (NMR) or X-ray crystallography, and a binding "pocket" for phosphotyrosine has been identified (33–35). However, the structural basis for the specificity of the interaction between phosphotyrosine-containing sequences and SH2 domains has not been defined. It seems likely that the sequences on the carboxyl-terminal side of the phosphotyrosine interact with a specific β-sheet region that is near the phosphotyrosine-binding site, although this has yet to be proved.

A large number of proteins containing SH2 domains have been identified (29, 30). The specific sequences that these proteins recognize and bind to have been identified for PI3-kinase, PLC-γ, and GAP. Some of the SH2-containing proteins that bind RTKs have known enzymatic activities. For

example, PLC-γ and GAP are known to utilize phosphatidylinositol and p21ras in its GTP-bound form, respectively, as their substrates. Another SH2 protein, termed corkscrew, is a tyrosine phosphatase and is thought to play a role in signal transduction by the *Drosophila* RTK encoded by the *torso* gene (17). Other SH2-containing proteins appear to serve as "linkers/adapters" between receptors and enzymes. For example, the PI3-kinase catalytic subunit, p110 (36) is linked to the PDGF receptor through the p85 subunit, which consists of two SH2 domains and an SH3 domain [Figure 2; (37–39)]. Proteins including c-crk, nck, vav, and sem-5 (GRB2 represents the human homologue of sem-5) consist almost entirely of SH2 and SH3 domains and are also candidate linking proteins between RTKs and effector molecules further downstream in the signal transduction pathways (10, 30, 40–44). Another protein, SHC, includes an SH2 domain linked to a glycine/proline-rich region. The SHC protein appears to interact with the EGF receptor as well as a number of other RTKs and also may bind the GRB2 protein (sem-5) (45). Therefore, complexes consisting of two or more SH2 proteins may form in response to activation of the tyrosine kinase.

The functional consequences of the associations between receptors and signalling molecules are not entirely clear. It is possible that these associations simply serve to localize the signalling molecules to sites near their substrates. Many of these signalling molecules, for example PLC-γ or PI3-kinase, utilize substrates in the plasma membrane. Another possible function of the complex of RTKs and signalling molecules is the positioning of signalling molecules so that they are better substrates of the tyrosine kinase domains. The affinities of the interactions between RTKs and signalling molecules are in many cases extremely high, with dissociation constants in the subnanomolar range. By contrast, the K_ms for the phosphorylation of substrates by tyrosine kinase catalytic domains are closer to the micromolar range. Therefore, by forming high-affinity binding sites, low-abundance substrates can be phosphorylated more readily. In addition, phosphorylation of substrates may modify the conformation and activities of these molecules. Recently it has been shown that tyrosine phosphorylation of PLC-γ regulates its enzymatic activity (46). Therefore, it seems likely that the tyrosine kinase activity of RTKs has two functions: first, it is responsible for creating high-affinity binding sites for signalling molecules and second, the tyrosine kinase modifies the signalling molecule by phosphorylation.

Mapping Intracellular Signalling Cascades By Mutagenesis

One of the goals of studies on the transduction of signals by growth factor receptors has been to determine which of the early biochemical responses to growth factors are important in cellular responses such as proliferation, alterations in gene expression, or changes in cell shape and locomotion.

Mutating tyrosine residues on RTKs that are responsible for binding to specific molecules provides an approach for assessing the roles of signalling molecules in these biochemical and cellular responses to growth factors. For example, when the two PI3-kinase binding sites on the PDGF β-receptor are mutated and the mutant receptor is expressed in epithelial cells, PDGF-stimulated mitogenesis is abrogated even though accumulation of inositol phosphates, influx of calcium, and tyrosine phosphorylation of GAP are unaffected (21, 22). In cells expressing this mutant receptor, PDGF is unable to "activate" p21ras (assessed by measuring the ratio of GTP to GDP bound to p21ras), and is also unable to stimulate Raf-1 and ERK kinases (47). Therefore, one can conclude that PI3-kinase is essential for normal PDGF-induced mitogenesis, and that p21ras, Raf-1, and ERK kinases act downstream of PI3-kinase. By contrast, PLC-γ activation by PDGF does not require PI3-kinase. In cells expressing the PDGF β-receptor mutant that lacks the GAP-binding site, PDGF stimulation of PI3-kinase, p21ras, and DNA synthesis is normal, implying that GAP is unimportant in these processes (21, 22). When the PLC-γ-binding sites on the PDGF receptor are eliminated, PI3-kinase activation and GAP phosphorylation are normal but the mitogenic response to PDGF in epithelial cells is reduced. From these studies one can conclude that the PI3-kinase-dependent pathway and a PLC-γ-dependent pathway are required for normal PDGF-stimulated mitogenesis in epithelial cells that express transfected PDGF receptors. It is not yet clear whether these results can be extrapolated to the function of the PDGF receptor in cell types that normally express the receptor.

Recently the approach of selectively eliminating a signalling pathway by point mutation of a receptor has been particularly useful in demonstrating that PLC-γ binds to tyrosine 766 of the FGF receptor [see section in this chapter on FGF receptors; (48, 49)]. By extending this approach to other receptors and signalling molecules, it should be possible to elucidate the network of intracellular reactions that mediate the pleiotropic actions of growth factors.

p21Ras as a Mediator of Receptor Tyrosine Kinase Signals

Many studies have suggested that the p21 proteins encoded by the ras family of proto-oncogenes play crucial roles in mediating cellular proliferation stimulated by growth factors. The p21ras proto-oncogenes were discovered as the cellular homologs of the transforming genes of Harvey (Ha) and Kirstein (Ki) sarcoma viruses. Three closely related ras proteins (80% homology), Ha, Ki, and N, are found in most mammalian cells and are members of an expanding family of ras-like proteins. Ras genes are highly conserved and have also been identified in yeast, *Drosophila*, *C. elegans*, and *Dictyostelium discoideum* (50–52).

Several studies have allocated a central role for ras in growth factor-mediated signal transduction. Mulcahy et al (53) showed that microinjection of anti-ras monoclonal antibodies into mammalian fibroblasts made those cells refractory to proliferation in response to serum and growth factors. Dominant negative mutants of ras also caused this effect (54). Nerve growth factor–mediated differentiation of PC12 cells and insulin-induced maturation of oocytes can also be blocked by ras antibodies or dominant negative ras mutants (55–58).

The proteins encoded by the *Ha*, *Ki*, and *N ras* genes are 21 kDa, and are posttranslationally modified at their carboxyl terminus to allow anchorage to the inner surface of the plasma membrane. Ras proteins are GTPases and cycle between a conformation that is active, GTP-bound, or inactive, GDP-bound. Activating point mutations of ras keep the protein predominantly in its active GTP-bound conformation (50–52).

In untransformed cells, the basal level of activated ras is only a few percent of the total ras protein. In response to growth factors and cytokines, there is an increase in the ratio of GTP to GDP bound to ras (corresponding to a ratio of active to inactive state) (50, 59–62). RasGTP is able to couple to downstream effectors and transduce the signals necessary for proliferation or differentiation. The signalling pathway(s) that are responsible for generating the GTP-bound form of ras in higher eukaryotes are at present undelineated. However, two groups of proteins have recently been identified that regulate the ratio of active versus inactive ras. The first group is composed of GAP and the neurofibromin gene product, proteins that greatly accelerate the intrinsic GTPase activity of ras (50). Constitutively activated mutant ras proteins are resistant to the action of GAP, giving rise to a phenotype of uncontrolled cellular proliferation.

GAP becomes physically associated with two proteins, p62 and p190, following stimulation with EGF (63). The p62 protein has homology with two types of RNA-binding proteins (64), while p190 has homologies with G proteins, n-chimerin, rho-GAP, and a transcriptional repressor (65). At present it is unclear how GAP, p62, and p190 connect with the network of cellular pathways that lead to mitogenesis.

The second group of proteins that influence the state of ras are the nucleotide exchange factors or guanine nucleotide releasing proteins. GNRPs facilitate the activation of ras by removing bound GDP from inactive ras. Reactivation of ras is dependent on GNRPs because GDP has a low rate of dissociation from ras. Thus, GNRPs can be viewed as activators, and GAPs as down-regulators of ras activity (50, 66). However, the situation is likely to be more complex, since several studies suggest that GAP may be coupled to a signalling function of ras and therefore be an effector of ras action. In one

such study, GAP was shown to act downstream of ras in isolated atrial potassium channels (67). Interestingly, this effect of GAP requires its SH2/SH3 domain, but not its catalytic domain.

It is likely that growth factor receptors change the nucleotide state of ras by activation of guanine nucleotide exchange proteins (see above). The most compelling evidence for the importance of exchange factors in signal transduction comes from genetic studies in yeast and *Drosophila*. In yeast, CDC25, SDC25, and ste6 have been identified as exchange factors for Ras (50, 68). In *Drosophila*, differentiation of R7 photoreceptor cells involves signal transduction through a RTK called sevenless. Genetic analyses have identified four additional proteins that are required for signalling by sevenless, including ras1 and son of sevenless (SOS). SOS is closely related to the yeast nucleotide exchange factors and is postulated to link sevenless with ras1 (11–13).

Given the homologies between yeast and *Drosophila* exchange proteins, several groups designed PCR primers to isolate the vertebrate homologs (69–71). One group isolated a clone corresponding to a protein of 140 kDa with a homology of 30% over 300 amino acids to the carboxyl-terminal portion of CDC25 (70). In addition, the amino-terminal half displayed substantial homology with the *dbl* oncogene product. dbl also shares some homologies with the vav protein (72). The dbl protein is distinct from CDC25 and SOS and catalyzes nucleotide exchange from CDC42Hs (73), a ras-like protein involved in regulating cell shape. CDC42Hs is similar to rac and rho, two ras-like proteins that are activated by growth factors and play a role in cytoskeletal changes (74, 75). An additional class of nucleotide exchange factors (small-molecular-weight G proteins, smgs) has also been isolated by Takai's group (76).

Other proteins that may link growth factors with ras have been identified in genetic studies with *C. elegans*. Let-60, which has homology with ras, plays a key role in vulval development with the participation of two other gene products, sem-5 and let-23 (EGFR), (7, 9, 10). Recently, GRB2, a human protein homologous to sem-5, has been cloned (77). GRB2 has two SH3 domains and one SH2 domain. The latter domain is involved in forming a complex with an activated RTK. Coinjection of GRB2 and c-Ha ras into fibroblasts produced a potent mitogenic effect not seen upon injection of each protein individually. Perhaps GRB2 controls ras activity through a guanine exchange protein (77).

Important issues to be resolved in the near future are (*a*) How do growth factors regulate ras activity, and (*b*) Which class(es) of guanine nucleotide exchange proteins are involved in growth factor-mediated changes in ras activity? Finally, it will be important to decipher the role of proteins analogous to sem-5 in the activation of ras.

SUBFAMILIES OF RECEPTOR TYROSINE KINASES

The Epidermal Growth Factor Receptor Family

The receptor for epidermal growth factor (EGFR) is a 170-kDa transmembrane tyrosine kinase that is expressed on a wide variety of cell types. Other members in the EGF receptor family (Class I, Figure 1) include p185neu tyrosine kinase (also referred to as erbB-2 or Her2) and erbB-3. All three receptors have two cysteine-rich clusters in the extracellular region and an uninterrupted tyrosine kinase domain in the cytoplasmic region (78, 79).

A family of ligands have been identified that bind with high affinity to the EGFR and elicit a mitogenic response in EGF-sensitive cells. Members of this family include TGF-α (80), the pox virus growth factors, amphiregulin, and heparin-binding EGF-like growth factor (78). A nematode homolog of EGF, lin-3, has also been identified (7). Recently, a ligand for p185neu was purified and cloned. Based on its ability to induce phenotypic differentiation in certain cells, the ligand was named "neu differentiation factor" (NDF) (81). Huang & Huang (82) have purified a ligand for p185neu, termed "neu/erbB ligand growth factor" (NEL-GF), which is structurally and biologically distinct from NDF.

The interaction of ligand with the EGFR or p185neu results in receptor dimerization and autophosphorylation (19, 79). For the EGFR, tyrosines 992, 1068, 1148, 1173, and 1186 in the C-tail are sites of autophosphorylation (83–86). Some studies have shown that autophosphorylation enhances tyrosine kinase activity, whereas others have shown that there is no effect (83, 86). Point mutation of lysine 721, the ATP-binding site, abolishes kinase activity and receptor-mediated calcium flux, pH change, transcriptional activation, and cell division (87, 88). However, in an additional study, a kinase-defective EGFR was able to mediate activation of ERK (89).

It is controversial whether the kinase activity of the EGFR is required for ligand-induced receptor downregulation, a process that involves receptor internalization, endocytotic targeting, and lysosomal degradation (90). An 18-amino-acid motif in the C-tail domain of the EGFR appears to be required both for receptor downregulation (internalization) and for EGF-mediated increases in cytosolic calcium (91).

A number of signalling molecules associate with activated EGF receptors. Treatment of cells with EGF leads to increased phosphatidylinositol turnover, because of a high-affinity interaction between tyrosine 992 in the EGFR with PLC-γ (92, 93). The PLC-γ enzyme also becomes phosphorylated on tyrosine residues (46). The exact role of PI turnover in EGF-mediated cell growth remains to be elucidated. Cochet et al have shown that PI4- and PI5-kinase also associate with activated EGFR (94).

SHC proteins of molecular weights 46,000, 52,000, and 66,000 have been found to associate with activated EGFRs in vivo, most likely through their SH2 domain (45). The SHC proteins also become phosphorylated on tyrosine residues. SHC proteins consist of an SH2 domain and a proline/glycine-rich domain, and are devoid of any known catalytic domain. Therefore, they may serve a role as "linker/adapter" proteins similar to that of the p85 subunit of the PI3-kinase (see section above on the interactions between RTKs and signalling molecules).

Although a physical complex between GAP and the EGFR has not been shown, GAP and its associated proteins p62 and p190 become tyrosine and serine phosphorylated following stimulation with EGF (63), and in vitro studies have detected complexes between isolated GAP SH2 domains and the C-tail domain of the EGFR (95). Activation of PI3-kinase by EGFR differs depending on the cell type. In PC12 cells, PI3-kinase is activated by EGFR with kinetics comparable to those of other growth factor receptors (96).

EGFR counterparts have been identified in *Drosophila* and *C. elegans*. Genetic analyses of *Drosophila* EGFR (DER) have revealed a wide variety of roles for this protein, including determination of cell fate and eye development (97). As described in a previous section, vulval development in *C. elegans* requires a hierarchical signal transduction pathway in which the EGFR homolog, let-23, signals through a ras homolog (see section on GENETIC SYSTEMS).

The Insulin Receptor Family

The insulin receptor (IR) is the prototype for a family of RTKs whose distinctive structural feature is to function as a heterotetrameric species of two α and two β subunits. The extracellular ligand-binding subunit is disulfide-linked to the transmembrane β subunit, which contains the cytoplasmic tyrosine kinase domain (79, 98, 99). Other members of the IR tyrosine kinase family include the receptor for insulin-like growth factor-1 (IGF-1R) (100) and the insulin-related receptor (IRR) (101, 102), whose ligand(s) are at present unidentified. Although there is significant homology between insulin/IGF-1 and IGF-2, the receptor for IGF-2 lacks any homology with the receptors for insulin and IGF-1, and indeed has been shown to be identical with the cation-independent mannose 6-phosphate receptor (103). The biological role of IGF-2 binding to this receptor is unresolved.

When insulin binds to its receptor, a variety of responses is elicited, which can be divided into metabolic effects and growth-promoting effects. Metabolic effects include glucose transport and glycogen synthesis (99, 104, 105). Recently, Dent et al (106) have shown that an insulin-activated protein kinase phosphorylates protein phosphatase type 1, which when activated increases the enzymatic activity of glycogen synthase by dephosphorylating a series of

serine residues in the glycogen synthase molecule. Other insulin-mediated metabolic effects include lipogenesis, lipolysis, and protein synthesis. Growth-promoting effects include DNA synthesis, cell division, and differentiation.

Like other growth factors, insulin and IGF-1 interact with the extracellular regions of their respective receptors. Despite conservation of structural elements within the extracellular domain of the two receptors, insulin and IGF-1 bind to distinct regions of the respective α subunits (107). Ligand-binding stimulates receptor autophosphorylation on the β subunit (108) and activates the kinase towards cytoplasmic signalling molecules (109, 110). Several studies have shown that insulin binding may enable each β subunit to phosphorylate itself in an intramolecular *cis* reaction (111). Other studies, however, indicate that phosphorylation of the β subunit occurs via an intermolecular transphosphorylation mechanism (112). If the mechanism is the latter, then this may explain the dominant negative phenotype observed in patients suffering insulin resistance due to the presence of a kinase-defective insulin receptor allele.

The kinase activity of the insulin receptor is essential for insulin-mediated cellular responses (108). The major sites of tyrosine phosphorylation in the human β subunit have been mapped to two regions, the C-tail domain (tyrosines 1316 and 1322) and the region encompassing tyrosines 1146, 1150, and 1151 [numbering according to Ullrich et al (113)]. In two studies, removal of tyrosines 1316 and 1322 augmented insulin-dependent mitogenesis in rat-1 fibroblasts (114, 115). In a separate study, removal of the last 43 amino acids of the β subunit (including tyrosines 1316 and 1322) produced an insulin receptor that functioned normally in Chinese hamster ovary cells (116). Phosphorylation of tyrosines 1146, 1150, and 1151 is, on the other hand, necessary to activate the kinase activity of the β subunit (108, 117, 118). Mutation of tyrosines 1161 and 1162 (equivalent to tyrosines 1150 and 1151) (119) severely reduced insulin-stimulated autophosphorylation.

An interesting aspect of insulin receptor signalling is that tyrosines in the cytoplasmic region nearest the membrane-spanning sequence (the juxtamembrane domain) appear to be important in mediating biological responses. Mutation of tyrosine 960 in the juxtamembrane domain had no effect on insulin-mediated receptor autophosphorylation or kinase activity (120, 121). However, this mutant receptor was biologically inactive (120) or elicited a reduced biological response (121), which correlated with significantly impaired tyrosine phosphorylation of IRS-1 (pp185), a major insulin receptor substrate (120, 122).

The IRS-1 molecule contains more than 10 potential tyrosine phosphorylation sites. Six of these are within the motif Y-M-X-M. A form of this motif, Y-X-X-M, is present in all proteins that are known to associate with PI3-kinase

(122–125) (see section above on interactions between signalling molecules and receptors). Indeed, PI3-kinase activity is found to be associated with IRS-1 immunoprecipitates and is increased following insulin treatment (122). Thus, by analogy with the PDGF receptor, an SH2 domain of p85 could associate with one or more of these motifs on IRS-1 after it has become tyrosine phosphorylated. IRS-1 might act as a docking protein for PI3-kinase or other signalling molecules important for insulin action. The unique aspect of this model is that the PI3-kinase binds to a substrate of the insulin receptor kinase rather than to the receptor itself (126). It is not clear whether binding of IRS-1 to p85 is sufficient to stimulate PI3-kinase enzymatic activity or whether the PI3-kinase needs to be modified, by phosphorylation or by some other means (127).

Other proteins that become phosphorylated on tyrosine in response to insulin include fatty acid binding protein (15 kDa), ecto ATPase (120 kDa), and ERK kinases (42 kDa) (109). The exact roles of these proteins in signal transduction mediated by the insulin receptor are unclear. In addition to tyrosine phosphorylation, insulin promotes serine/threonine phosphorylation of the β subunit and a number of intracellular substrates including Raf-1 (128, 129).

The PDGF/MCSF-1/Steel Receptor Family

This receptor family includes the platelet-derived growth factor (PDGF) α- and β-receptors, the macrophage colony stimulating factor-1 (MCSF-1) receptor, and the c-kit protein (a receptor for the steel ligand). The distinctive features of this group of receptors are that their extracellular regions consist of five domains that have primary sequences characteristic of immunoglobulin domains and their cytoplasmic regions contain a tyrosine kinase domain that is interrupted by a large insert sequence termed the "kinase insert" region (Figure 1). One function of the kinase insert region appears to be the presentation of phosphotyrosine-containing binding sites for signalling molecules (see section above on interactions between RTKs and signalling molecules).

THE PDGF RECEPTORS PDGF is a potent mitogen for smooth muscle cells, glial cells, oligodendrocyte progenitor cells, fibroblasts, and selected types of endothelial cells (130). PDGF has been found in at least three dimeric forms, each consisting of disulfide-linked A chains or B chains in either homodimeric (AA or BB) or heterodimeric (AB) combinations. There are two known types of PDGF receptor, termed PDGFαR and PDGFβR. The α- and β-receptors are quite similar in sequence and have the same configuration of structural domains. The major known differences between the two types of PDGF receptor are the ligand-binding specificities and the pattern of expression in tissues. Both the α- and β-receptors mediate PDGF-stimulated

mitogenesis, but only the β-receptor appears to mediate ligand-induced changes in cytoskeleton (131). The patterns of proteins phosphorylated by the α- and β-receptors are similar, though not identical.

Upon binding PDGF, PDGF receptors form noncovalent dimers. The pattern of dimer formation in the presence of different divalent forms of PDGF has suggested that the A-chain ligand binds to only the PDGFαR, whereas the B-chain ligand binds to both PDGFαR and PDGFβR. Therefore, the type of receptor dimer (aa, bb, or ab) that is formed in response to ligand binding depends not only on the type of receptor expressed in a given cell, but also on the form of PDGF dimer (AA, BB, or AB) used to stimulate the cell. Recent studies have shown that truncated PDGF receptor mutants lacking receptor cytoplasmic and transmembrane domains form PDGF-dependent dimers in solution (132). Therefore, neither cytoplasmic nor transmembrane sequences are required for ligand-induced receptor dimerization. This finding was not anticipated, since the transmembrane sequences of PDGF β-receptors are highly conserved among different species.

The first evidence that signalling molecules bind directly to RTKs came from a study of the interaction of PDGF β-receptor with PI3-kinase. Subsequently it was shown that the PDGF receptor also binds PLC-γ, GAP, raf, and pp60c-src tyrosine kinase (133–138). The binding sites for PI3-kinase, GAP, and PLC-γ on the PDGF receptor have been localized and have suggested the model of signal transduction shown in Figure 2 (see section above on interaction between RTKs and signalling molecules for a detailed discussion of these binding sites).

THE MCSF-1 RECEPTOR The receptor for macrophage colony stimulating factor-1 (MCSF-1R) was first identified as the cellular counterpart of v-fms, the transforming oncogene of the feline McDonough sarcoma virus (139). MCSF-1 plays an important role in the regulation of proliferation, differentiation, and survival of macrophages. The binding of MCSF-1 to its receptor activates the receptor tyrosine kinase, resulting in autophosphorylation and tyrosine phosphorylation of putative cytoplasmic signalling molecules. MCSF-1 treatment also results in receptor internalization and degradation via two distinct mechanisms, one involving receptor tyrosine kinase activity and the other involving the activation of a protease that acts on the extracellular domain of the receptor (140).

There are several potential sites of tyrosine autophosphorylation on the MCSF-1 receptor. Three of these lie within the kinase insert domain and a fourth, conserved in all protein tyrosine kinases, is outside the kinase insert domain (141). Deletion of the kinase insert domain of human MCSF-1R produced a receptor that was unable to activate PI3-kinase but could still induce cellular proliferation (142, 143). Indeed, one tyrosine in the kinase

insert region (721 in human) fits the consensus sequence for PI3-kinase binding: Y-V-E-M. Mutation of this tyrosine to phenylalanine abolished ligand-dependent activation of PI3-kinase (144). Whether other SH2-containing signalling molecules associate with the other three phosphotyrosines on the MCSF-1R remains to be determined.

Mutation of tyrosine 809 (human), which lies outside the kinase insert region, produced a receptor with an impaired mitogenic response even though tyrosine kinase activity, activation of PI3-kinase, and induction of c-fos and junB were normal. The mutant receptor also fails to mediate normal regulation of c-myc and other early response genes (145). Additional studies have shown that mutation of tyrosine 706 (mouse) affects c-fos and c-jun induction (146). In contrast to the PDGF receptor, the MCSF-1R does not appear to associate with PLC-γ or GAP and fails to stimulate PI turnover (147).

THE STEEL RECEPTOR The steel receptor (also called the c-kit protein) is a transmembrane RTK whose ligand has been variously referred to as steel factor (SF), stem cell factor, mast-cell growth factor, and kit ligand (148). The steel ligand is important in the development of melanocytes and germ cells, and also plays a role in hematopoiesis. The binding of steel factor to its receptor activates the receptor tyrosine kinase activity and stimulates the activity of cytoplasmic signalling molecules (149–151). One signalling molecule that becomes activated is PI3-kinase. In addition, treatment of cells with steel ligand leads to an increase in the ratio of GTP/GDP that is bound to ras (152).

The Vascular Endothelial Cell Growth Factor Receptor Family

A receptor for vascular endothelial cell growth factor (VEGF) has recently been identified that contains seven Ig-like domains and a cytoplasmic tyrosine kinase domain that is interrupted by a large kinase insert domain (153). Additional distinct members of this subfamily of RTKs have also recently been cloned (154). The VEGF receptor is remarkable in that it is expressed exclusively in endothelial cells (155). VEGF mediates mitogenesis in endothelial cells and stimulates an increase in vascular permeability, presumably by affecting tight junctions between endothelial cells (156). Although the VEGF receptor is a tyrosine kinase, little is known about VEGF receptor-mediated signalling systems.

The Hepatocyte Growth Factor Receptor

Hepatocyte growth factor (HGF) and scatter factor (SF) are identical proteins (157) that elicit diverse biological responses in epithelial cells, including mitogenesis, dissociation of epithelial sheets, and stimulation of cell motility (158). The HGF/SF ligand binds to the met proto-oncogene. This receptor is

a heterodimeric transmembrane tyrosine kinase composed of an entirely extracellular α subunit (50 kDa) that is disulfide-linked to the membrane-spanning β subunit (145 kDa). Binding activates the receptor tyrosine kinase activity, allowing in vitro association with several signalling molecules including PLC-γ, GAP, and src-related kinases, and with PI3-kinase both in vitro and in vivo (158a).

The Neurotrophin Receptor Family

The neurotrophins are a family of ligands that play important roles in the growth, differentiation, and survival of neurons. In non-neuronal systems, neurotrophins also have the capacity to stimulate cellular proliferation. Currently there are five known members in this family, including nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), neurotrophin-4 (NT-4), and neurotrophin-5 (NT-5) (159).

There are both high-affinity ($Kd = 10^{-11}$ M) and low-affinity ($Kd = 10^{-9}$ M) binding sites for neurotrophins on responsive cell types (160, 161). Biological responses to a specific neurotrophin appear to be mediated through binding to the high-affinity receptor population. Although high- and low-affinity sites are interconvertible, the exact biochemical basis for high- and low-affinity sites remains unresolved (see discussion below).

Two distinct classes of proteins have been identified as receptors for neurotrophins. One of these classes is represented by the $p75^{LNGFR}$ protein (also referred to as $gp80^{LNGFR}$ or LNGFR) (162, 163). The $p75^{LNGFR}$ protein is a 75–80-kDa membrane glycoprotein that is highly conserved across species and is broadly expressed in neuronal and non-neuronal tissues (164). The extracellular region of $p75^{LNGFR}$ contains a cysteine-rich domain that bears some resemblance to cysteine-rich domains in several other proteins (e.g. TNF receptor) (159). The intracellular domain of $p75^{LNGFR}$ (155 amino acids in the human protein) is not homologous to any known protein and has no known enzymatic function. When $p75^{LNGFR}$ is expressed in fibroblasts it binds all of the known neurotrophins with low affinity (*K*ds approximately 10^{-9} M).

The second class of neurotrophin receptors are encoded by the *trk* (also referred to as *trk*A) (165), *trk*B (166, 167), and *trk*C (168) genes. In contrast to $p75^{LNGFR}$, the cytoplasmic regions of $p140^{trk}$, $p140^{trkB}$, and $p145^{trkC}$ contain tyrosine kinase catalytic domains. The extracellular regions of the mammalian trk proteins do not contain any obvious structural elements, although it has been suggested that they may contain Ig-like domains and fibronectin type III (FNIII) domains (169). The extracellular region of *Drosophila* trk appears to contain six consensus Ig-like domains (170).

Each member of the trk family can bind at least one member of the neurotrophin family (171–178). Ligand binding activates the tyrosine kinase

activity of the receptor molecule. The $p140^{trk}$ protein binds and becomes activated by NGF, NT-3, NT-4, and NT-5, but not BDNF. The $p140^{trkB}$ protein binds and becomes activated by BDNF, NT-3, NT-4, and NT-5, but not NGF. The $p145^{trkC}$ protein binds and becomes activated by NT-3, but not by NGF or BDNF.

The functional relationship between $p75^{LNGFR}$ and the trk family of receptor proteins remains unclear. A substantial body of evidence demonstrates that trk receptors can mediate biological responses to neurotrophins in the absence of $p75^{LNGFR}$. On the other hand, other evidence indicates that $p75^{LNGFR}$ plays some role in signal transduction. Several recent reviews discuss the potential role of both receptor classes in signal transduction pathways (159, 160, 179, 180).

Signalling pathways that are induced by neurotrophins have been studied most extensively in the PC12 cell line (181). Treatment of PC12 cells with NGF leads to rapid activation (tyrosine kinase activity) and autophosphorylation of the $p140^{trk}$ receptor, as well as rapid tyrosine phosphorylation and association of PLC-γ with $p140^{trk}$ (182, 183). Some groups have reported that NGF treatment also leads to increased levels of intracellular cAMP (181). Others, however, have not observed an increase in cAMP levels (181).

Additional effects of NGF stimulation include membrane ruffling, increased intracellular Ca^{2+} levels, increased PI turnover, increased transcription of c-fos and c-jun, and increased phosphorylation of a number of intracellular proteins (181). Increases in phosphorylation may take the form of serine, threonine, or tyrosine phosphorylation, and frequently lead to an increase in the activity of the phosphorylated protein. Proteins whose enzymatic activities become enhanced as a result of increased phosphorylation following NGF treatment include tyrosine hydroxylase, ERK 1, ERK 2, S6 ribosomal kinase, and Raf-1 (184–187). Additional proteins that become phosphorylated include synapsin I, peripherin, and the S6 ribosomal subunit (181). Gomez & Cohen (187) have characterized an activity (MAP kinase kinase, or MAPKK) in NGF-stimulated PC12 cells that phosphorylates ERK 1 and ERK 2 on serine, threonine, and tyrosine residues. The activity of their MAPKK is dependent on serine/threonine phosphorylation.

Several experiments have shown that ras is involved in the signalling pathways that lead to neurite outgrowth. Expression of oncogenic ras in PC12 cells leads to activation of ERK 1 and ERK 2, hyperphosphorylation of Raf-1, and ultimately neurite outgrowth (57, 58). In contrast, microinjection of anti-ras antibodies blocks NGF-dependent neurite outgrowth in PC12 cells (56). Similarly, expression of a dominant negative form of ras also blocks NGF responsiveness (188). Expression of dominant negative ras does not inhibit autophosphorylation of $p140^{trk}$ or association of PLC-γ with the receptor, indicating that ras is located further downstream in the signalling

pathway (57). On the other hand, dominant negative ras antagonizes NGF-dependent activation of ERK 1 and ERK 2, as well as hyperphosphorylation of Raf-1 (57, 58). This means that ras is located upstream of these important serine/threonine kinases. Also, Qui et al (189) have shown that isoprenylation of ras is important for induction of neurite outgrowth by oncogenic ras, but is not important for normal NGF-dependent neurite outgrowth. This indicates that the oncogenic and proto-oncogenic forms of ras may utilize distinct pathways to elicit neurite outgrowth.

Kremer et al (190) have reported that microinjection of anti-src antibodies into PC12 cells blocks NGF-dependent neuronal differentiation. Furthermore, whereas microinjection of anti-ras blocks v-src-induced neurite outgrowth, microinjection of anti-src does not inhibit neurite outgrowth induced by oncogenic ras. These results indicate that c-src may be upstream of ras.

It is interesting that many of the signalling molecules involved in pathways leading to neurite outgrowth in PC12 cells have previously been implicated in pathways leading to mitogenesis in other cell types. Thus, src, ras, ERK 1, ERK 2, Raf-1, and S6 ribosomal kinase can become activated in cells induced to undergo either proliferation of differentiation. How the activation of these proteins leads to proliferation in one cell type and differentiation in another is not understood.

The Fibroblast Growth Factor Receptor Family

Fibroblast growth factors (FGFs) mediate a diverse array of biological responses, including proliferation, differentiation, and survival of cells (191, 192). FGFs also have the capacity to induce angiogenesis in a variety of organisms (193) and to induce mesoderm formation in *Xenopus* embryos (194, 195). Currently, there are seven known members in the FGF family, including acidic FGF (aFGF), basic FGF (bFGF), the product of the *int-2* oncogene, the product of the *hst* oncogene (also referred to as Kaposi sarcoma FGF), FGF 5, FGF 6, and keratinocyte growth factor (KGF) (191, 196–198).

Four distinct genes, *FGFR1*, *FGFR2*, *FGFR3*, and *FGFR4* (see references 199, 200 for a discussion of nomenclature), encoding cell surface FGF receptors have been identified (201–204). The products of these genes are structurally related, exhibit tyrosine kinase activity, and are differentially expressed in a variety of tissues during mouse embryogenesis. FGFs also bind to heparin sulfate proteoglycan molecules located on the cell surface or in the extracellular matrix (205). Recent evidence indicates that the binding of FGFs to heparin sulfate proteoglycans plays a role in potentiating the binding of FGFs to the tyrosine kinase receptors (206). This discussion focuses on the tyrosine kinase forms of the FGF receptor.

The prototypical FGF receptor contains a single membrane-spanning

domain. The extracellular region of the receptor contains three consensus Ig-like domains. Also, between Ig domains I and II is a short domain referred to as the acid box domain. In the FGFR1 protein, this domain contains a core sequence of eight consecutive acidic residues. The cytoplasmic region of the receptor contains the tyrosine kinase catalytic domain. A short (14 amino acids) kinase insert sequence splits the kinase domain into two nearly equal halves. Following the tyrosine kinase domain is a C-tail domain of approximately 55 to 65 amino acids.

In the case of the *FGFR1* and *FGFR2* genes, multiple forms of the FGF receptor are generated via alternative splicing (202, 207–212). Alternative splicing generates FGFR1 and FGFR2 forms with or without Ig domain I, and FGFR2 forms with or without the acid box domain. Removal of Ig domain I or the acid box domain does not affect the binding affinity of FGFR1 or FGFR2 for either aFGF or bFGF, suggesting that these domains may play a role unrelated to ligand binding (202, 210, 211, 213).

The second half of Ig domain III represents another site of alternative splicing in both FGFR1 and FGFR2 (207, 214). For FGFR1, three alternative exons can code for the second half of Ig domain III: exons IIIa, IIIb, and IIIc (214). Expression of the IIIa exon generates a secreted form of FGFR1, whereas expression of either the IIIb or the IIIc exon generates a membrane-spanning form of the receptor. The in vivo expression pattern of the different exons is regulated in a tissue-specific fashion (215). For FGFR2, forms containing IIIb-type or IIIc-type sequences have also been identified, although there is no evidence for a IIIa-type exon in FGFR2 (202, 207, 210). Finally, alternative splicing appears to be responsible for generating three distinct C-tail domains in FGFR2 (202, 207, 216).

Binding studies have shown that alternative splicing in the third Ig domain of FGFR1 and FGFR2 is important for determining ligand-binding specificities (210, 213, 215). Hence, aFGF binds with high affinity to receptor forms containing IIIb- or IIIc-type sequences, but with much lower relative affinity to the receptor form containing IIIa-type sequence. Basic FGF, on the other hand, binds with high affinity to receptor forms containing IIIc-type sequences, but with much lower affinity to receptor forms containing IIIb-type sequences. Finally, in the case of FGFR2, KGF binds with high affinity to the receptor form containing IIIb-type sequence, but does not bind to the receptor form containing IIIc-type sequence.

Analogous FGF receptor forms derived from distinct FGF receptor genes also exhibit some similarities as well as some differences in binding properties (202–204, 211, 217, 218). For instance, comparison of the human FGF receptor proteins that contain three Ig domains and IIIc-type sequences reveals that aFGF binds with high affinity to all four gene products. Basic FGF, on the other hand, binds with high affinity to FGFR1 and FGFR2,

with low affinity to FGFR3, and does not bind to FGFR4. In addition, hst binds with high affinity to FGFR2, but with much lower relative affinity to FGFR1.

On the basis of the binding studies presented above it has been proposed that tissues can achieve selective responsiveness to individual members of the FGF family through either of two mechanisms: (*a*) tissue-specific alternative splicing in the third Ig domain, and/or (*b*) tissue-specific differential gene expression. Evidence from tissue localization studies of the different receptor forms indicates that both of these mechanisms probably occur in vivo (199).

The binding of FGFs to their receptors leads to activation of the receptor tyrosine kinase activity and autophosphorylation on tyrosine residues. Binding also induces receptor dimerization. Interestingly, both homodimeric and heterodimeric receptor species can be formed between FGFR1, FGFR2, and FGFR3 proteins (219, 220). Phosphorylation of the dimerized receptors appears to occur via an intermolecular transphosphorylation mechanism (220).

Stimulation with FGFs also leads to increased intracellular pH and Ca^{2+} levels, increased PI turnover, and increased phosphorylation of a number of intracellular proteins (191). Proteins that become phosphorylated in response to FGFs include PLC-γ, Raf-1, ERK 1 and ERK 2 kinases, and S6 ribosomal kinase (191, 221, 222). The enzymatic activities of these proteins are also increased. In addition, PLC-γ forms a stable complex with the FGF receptor following ligand stimulation.

The association of PLC-γ with the FGF receptor results from the recognition of a single phosphorylated tyrosine residue (766) in the C-tail domain of the receptor by the SH2 domain of PLC-γ (48, 49). Mutation of tyrosine 766 to phenylalanine generates a receptor protein that does not associate with or phosphorylate PLC-γ when expressed in L6 myoblasts or Chinese hamster ovary cells (48, 49). The mutant receptor also fails to mediate ligand-dependent PI turnover and increases in intracellular Ca^{2+} levels. The mutant receptor does however, autophosphorylate, mediate the phosphorylation of other cellular proteins, and mediate cellular proliferation. These results demonstrate that the ability of FGF to promote cellular proliferation in L6 cells and CHO cells is not dependent on PI hydrolysis or Ca^{2+} mobilization.

Recent studies using a dominant negative mutant of Raf-1 serine/threonine kinase have suggested that Raf-1, as well as kinases in the MAP/ERK kinase family, are important for FGF-induced formation of embryonic mesoderm (223). Several groups have also shown an involvement of ras in FGF receptor-dependent signalling pathways. Expression of dominant negative forms of ras inhibits FGF-dependent mitogenesis in NIH3T3 cells and mesoderm induction in *Xenopus* embryos (54, 224). Also, in a manner

similar to their action in NGF-treated PC12 cells, dominant negative forms of ras block the induction of neurite outgrowth in PC12 cells treated with FGF (188). It will be interesting to determine how the activation of ras by FGF leads to dramatically different phenotypes in different cell types.

The Eph-Like Receptor Tyrosine Kinases

The Eph-like proteins are the largest subfamily of RTKs. At least seven distinct genes have been identified that code for Eph-like proteins, and partial cDNA sequences indicate that there may be even more members in this subfamily. These genes encode proteins of approximately 130–135 kDa, including Eph (225), Eck (226), Elk (227), Eek (228), Erk (228), Cek4 (229) [Mek4 (229) and HEK (230) appear to be the murine and human homologs, respectively, of Cek4], and Cek5 (231).

The extracellular regions of the Eph-like proteins contain an amino-terminal cysteine-rich domain followed by two FNIII domains (225, 231). The presence of FNIII repeats in the Eph-like proteins has led to speculation that these receptors may also be involved in cell adhesion processes. The tyrosine kinase domains of the Eph-like receptors do not contain kinase insert sequences, and are followed by C-tail domains of approximately 90 to 100 amino acids.

To this date, no ligands have been identified that bind to any of the Eph-like proteins. This has seriously hampered the investigation of transduction pathways mediated by this large family of proteins.

The Axl Receptor Tyrosine Kinase

The *axl* gene encodes a 140-kDa protein that is related to the insulin and Eph-like receptor tyrosine kinase subfamilies (169). The extracellular region of Axl contains two amino-terminal Ig-like domains, followed by two FNIII domains. The ligand(s) for Axl have not yet been identified.

CONCLUDING REMARKS

While much has been learned regarding the identity of signalling molecules and pathways that are activated by RTKs, a number of questions remain unanswered. Two of these questions are depicted by Figure 3. Panel *A* indicates how a single growth factor, such as FGF, can act on a variety of different cell types leading to the activation of a number of common signalling molecules, such as ras. The phenotypic outcome in the different cell types, however, is dramatically different. Hence, it remains to be determined how the activation of ras by FGF or other growth factors leads to such diverse endpoints as mitogenesis, neurite outgrowth, or mesoderm induction. A second enigma is depicted in panel *B* of Figure 3. This panel indicates how different growth factors acting on the same cell type can

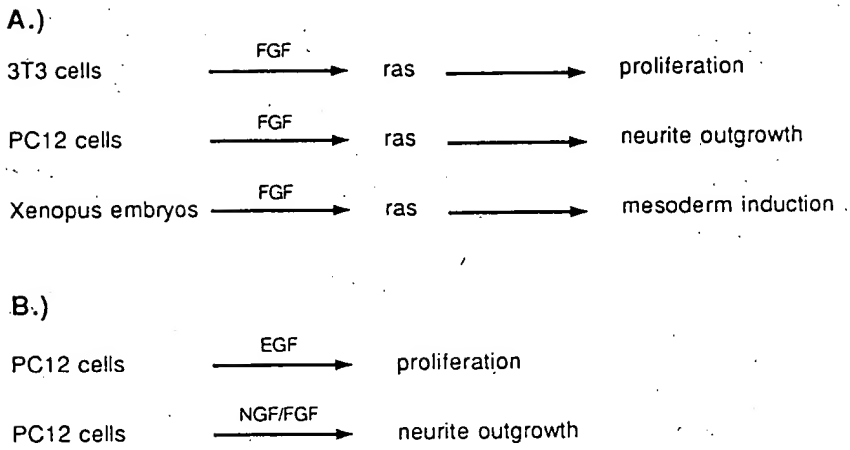

Figure 3 Enigmas in RTK-mediated signal transduction. The figure depicts two unanswered questions: (*A*) How does the activation of common signalling molecules, such as ras, by a single growth factor/RTK lead to different phenotypes in different cell types, and (*B*) How does signalling by different RTKs lead to different phenotypes in the same cell type?

induce different phenotypes. Hence, in PC12 cells, EGF induces proliferation, whereas NGF or FGF induce neurite outgrowth (159). This is in spite of the fact that EGF and NGF/FGF activate a number of common signalling molecules in these cells. Future studies will be needed to define precisely the quantitative and qualitative differences in the signalling pathways initiated by different RTKs.

A great deal also remains to be learned about the molecular architecture of signalling complexes and the network of interactions within specific signalling pathways. This will require identification of all of the molecules involved in a signalling pathway and determination of their order in the signalling cascade. Based on our current knowledge, newly identified molecules should include both enzymatic signalling molecules as well as "linker/adapter" molecules. Once the components of signalling pathways are identified, it will be important to define the regions of each molecule that are important for enzymatic activity, and for interaction with upstream or downstream molecules, as well as positive or negative regulators.

Finally, little is known about the mechanisms that are used to downregulate or turn off an activated pathway. Downregulation mechanisms will undoubtedly involve novel proteins, such as phosphatases, and novel feedback pathways. The elucidation of these pathways may provide valuable insight into a variety of pathological conditions, such as cancer, which involve abnormal cell growth.

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Due to the scope of this review and the limitations on space, we regret that we have not been able to cite all relevant literature on this topic.

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